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UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
18805-81106

Total Pages in this Submission
70

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

BIOSENSOR FOR USE IN TOXICITY ASSESSMENT AND PHARMACOLOGICAL SCREENING

and invented by:

JAMES J. HICKMAN, DOUGLAS G. KIRKPATRICK, AND DAVID A. STENGER

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☒ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: 09/513,720

Which is a:

☒ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: 09/372,568

Which is a:

☒ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: 09/236,684

which is continuation of prior application No. 09/109,481 which is a continuation of prior application No. 08/912,033 which claimed priority from provisional application No. 60/023,413.

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 60 pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☐ Cross References to Related Applications *(if applicable)*
   - c. ☒ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

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Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

   a. ☐ Formal Number of Sheets _____

   b. ☒ Informal Number of Sheets Five (5)

4. ☐ Oath or Declaration

   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted

   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*

   c. ☐ With Power of Attorney ☐ Without Power of Attorney

   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche *(Appendix)*

7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*

   a. ☐ Paper Copy

   b. ☐ Computer Readable Copy *(identical to computer copy)*

   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*

9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

   ☐ First Class ☒ Express Mail *(Specify Label No.)*: EL 773 915 763 US

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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Additional Enclosures *(please identify below):*

Request That Application Not Be Published Pursuant To 35 U.S.C. 122(b)(2)

17. ☐ Pursuant to 35 U.S.C. 122(b)(2), Applicant hereby requests that this patent application not be published pursuant to 35 U.S.C. 122(b)(1). Applicant hereby certifies that the invention disclosed in this application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication of applications 18 months after filing of the application.

Warning

An applicant who makes a request not to publish, but who subsequently files in a foreign country or under a multilateral international agreement specified in 35 U.S.C. 122(b)(2)(B)(i), must notify the Director of such filing not later than 45 days after the date of the filing of such foreign or international application. A failure of the applicant to provide such notice within the prescribed period shall result in the application being regarded as abandoned, unless it is shown to the satisfaction of the Director that the delay in submitting the notice was unintentional.

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Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	24	- 20 =	4	x $18.00	$72.00
Indep. Claims	3	- 3 =	0	x $80.00	$0.00
Multiple Dependent Claims (check if applicable) ☐					$0.00
				BASIC FEE	$710.00
OTHER FEE *(specify purpose)* _____					$0.00
				TOTAL FILING FEE	$782.00

☐ A check in the amount of to cover the filing fee is enclosed.

☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 50-1597 as described below. A duplicate copy of this sheet is enclosed.

- ☒ Charge the amount of $782.00 as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Signature

Dated: JUNE 12, 2001

Nisan A. Steinberg, Esq.
Reg. No. 40,345
SIDLEY AUSTIN BROWN & WOOD
555 West Fifth Street
Los Angeles, CA 90013
Tel: 213-896-6665
Fax: 213-896-6600

cc:

WHAT IS CLAIMED IS:

1. A biosensor comprising:

(a) a substrate in contact with a culture medium capable of supporting metabolism of at least one electrically excitable cell;

(b) a cell network composed of at least one of said electrically excitable cells, which cell has a predefined polarity on said substrate and is capable of producing a signal in response to a bioeffecting substance; and

(c) at least one signal transducer operably coupled to said cell network, which transducer is capable of detecting said signal produced in said cell network.

2. The biosensor of claim 1, wherein the signal produced by said cell network is an action potential, axonal wave potential, or dendritic wave potential.

3. The biosensor of claim 1, wherein the cell is a spinal cord cell, hippocampal cell, CNS excitatory cell line, or a cell line derived from stem cells.

4. The biosensor of claim 1, wherein said defined polarity is defined by a pattern of a self-assembled monolayer or biological macromolecule present on the surface of said substrate.

5. The biosensor of claim 1, wherein the transducer is a field effect transistor or a microelectrode.

6. The biosensor of claim 1, further comprising an insulating and/or barrier layer interposed between said at least one transducer and said culture medium, which insulating and/or barrier layer prevents direct contact between the culture medium and the transducer.

7. The biosensor of claim 6, wherein said insulating and/or barrier layer is selected from the group consisting of silica, silicon, germanium, gallium, arsenide, epoxy resin, polystyrene, polysulfone, aluminum, platinum, alumina, silicone, fluoropolymers, polyesters, acrylic copolymers, polyglactin, and polylactates.

8. The biosensor of claim 7, wherein the insulating and/or barrier layer comprises silica, silicon nitride, or silicon carbide.

9. The biosensor of claim 1, wherein said substrate has a patterned surface with at least one region thereon having an exposed surface of at least one cell adhesion promoter or cell adhesion inhibitor, which region is spatially related to the transducer so that a cell adhering to said region may be stimulated or detected by said transducer.

10. The biosensor of claim 9, wherein said cell adhesion promoter contains a terminal group selected from the group consisting of $-NHCH_2CH_2NHCH_2CH_2NH_2$, $-NHCH_2CH_2NH_2$, 11-aminoundecyl, 3-aminopropyl, 3-(1-aminopropoxy)-3,3-dimethyl-1-propenyl, 6-(aminohexyl)propyl,

N-(2-aminoethyl)-3-aminopropyl, $-(CH_2)_3-NH-(CH_2)_3-NH-(CH_2)_3$, Gly-Arg-Gly-Asp-Tyr-, and Gly-Tyr-Ile-Gly-Ser-Arg-Tyr.

11. The biosensor of claim 9, wherein said cell adhesion inhibitor is selected from the group consisting of tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-dimethylchlorosilane, tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane, tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-methyldichlorosilane, tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-triethoxysilane, (3,3,3-trifluoropropyl)trichlorosilane, (3,3,3-triflurorpropyl)methyldichlorosilane, (3,3,3-triflurorpropyl)-dimethylchlorosilane, (3,3,3-trifluoropropyl)methyldimethoxysilane, (3,3,3-trifluoropropyl)trimethoxysilane, (heptafluoroisopropoxy) propylmethyldichlorosilane, (3-pentafluorophenylpropyl) dimethylchlorosilane, polyethylene glycols, silanes having a branched or unbranched C_3-C_{40} alkyl terminus, phenyl groups, and inhibitory biological macromolecules.

12. The biosensor of claim 1, wherein a gigaohm seal is provided between the cell and the substrate.

13. The biosensor of claim 1, wherein a self-assembled monolayer is provided on the substrate in a predefined pattern, and the neuron is provided thereon.

14. The biosensor of claim 13, wherein a cell-repulsive surface is provided at the periphery of the self-assembled monolayer.

15. The biosensor of claim 13, wherein the self-assembled monolayer is composed of trimethoxysilylpropyl diethylene tetraamine (DETA).

16. The biosensor of claim 1, wherein said cell is a hippocampal neuron.

17. The biosensor of claim 1, wherein said transducer is capable of stimulating said electrically excitable cell.

18. The biosensor of claim 1, wherein the transducer is formed in the substrate.

19. A biosensor comprising:

a substrate;

first and second neurons provided on at least a portion of the substrate, said neurons each having a predefined polarity;

a first transducer adjacent one of said neurons and capable of detecting a signal therein; and

a second transducer adjacent one of said neurons and capable of stimulating or detecting a signal therein, said neurons being in synaptic relationship so that a signal established in one of the neurons is attenuated by the other neuron.

20. The biosensor of claim 19, further comprising a stimulator adjacent one of said first and second neurons, which upon stimulation is capable of affecting a signal established therein.

21. The biosensor of claim 19, wherein said first and second transducers are microelectrodes.

22. The biosensor of claim 19, wherein said first and second transducers are field effect transistors.

23. A method of detecting a bioeffecting substance in a test sample, comprising:

(a) providing said test sample and a biosensor as in claim 1, wherein at least one cell of said biosensor produces a detectable response to said bioeffecting substance;

(b) contacting the test sample with the biosensor;

(c) monitoring, with a transducer of the biosensor, a signal produced by said at least one cell in response to contacting said cell with the test sample; and

(d) correlating said signal to the presence or absence of said bioeffecting substance in the test sample.

24. The method of claim 23, wherein the biosensor comprises at least two transducers.